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Freezing fecal samples prior to DNA extraction affects the *Firmicutes* to *Bacteroidetes* ratio determined by downstream quantitative PCR analysis

Martin Iain Bahl*, Anders Bergström and Tine Rask Licht

National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark.

*mbah@food.dtu.dk

Freezing stool samples prior to DNA extraction and downstream analysis is widely used in metagenomic studies of the human microbiota but may affect the inferred community composition. In this study DNA was extracted either directly or following freeze storage of three homogenized human fecal samples using three different extraction methods. No consistent differences were observed in DNA yields between extractions on fresh and frozen samples, however differences were observed between extraction methods. Quantitative PCR analysis was subsequently performed on all DNA samples using six different primer pairs targeting 16S rRNA genes of significant bacterial groups and the community composition was evaluated by comparing specific ratios of the calculated abundances. In seven out of nine cases the *Firmicutes* to *Bacteroidetes* 16S rRNA gene ratio was significantly higher in fecal-samples that had been frozen compared to identical samples that had not. This effect was further supported by qPCR analysis of bacterial groups within these two phyla. The results demonstrate that storage conditions of fecal samples may adversely affect the determined *Firmicutes* to *Bacteroidetes* ratio, which is a frequently used biomarker in gut microbiology.